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WITNESS my hand this
Thirtieth day of April 2004

A handwritten signature in cursive script, reading 'J. Billingsley'.

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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AUSTRALIA

Patents Act 1990

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PROVISIONAL SPECIFICATION

Invention Title:

Cross-linked polysaccharide compositions

The invention is described in the following statement:

Technical Field

The present invention relates to biocompatible cross-linked polysaccharide compositions which are swellable, a process for preparing the compositions, and uses of the compositions in cosmetic, medical and pharmaceutical applications.

5

Background Art

Hyaluronic acid (HA) is a member of a class of polymers known as glycosaminoglycans. HA is a long chain linear polysaccharide and is usually present as the sodium salt which has a molecular formula of $(C_{14}H_{20}NNaO_{11})_n$ where n can vary according to the source, isolation procedure and method of determination. Molecular weights of up to 14×10^6 have been reported.

10

HA and its salts can be isolated from many sources including human umbilical cord, rooster combs and nearly all connective matrices of vertebrate organisms. HA is also a capsular component of bacteria such as streptococci as was shown by Kendall et al, (1937), Biochem Biophys Acta, 279,401-405; it may therefore also be obtained by fermentation methods, for example as described in US Patent No 5,411,874 (Fermentech Ltd).

15

HA is non-immunogenic and therefore has great potential in medicine. Because of its visco-elastic properties, HA having a high molecular weight (over 1 million) has been found to be particularly useful in a variety of clinical fields, including wound treatment, ophthalmic surgery and orthopaedic surgery. HA is also potentially useful in a variety of non-medical fields, such as cosmetic applications. However, the use of HA in certain of these applications is limited by the fact that following administration to humans, HA is readily degraded by enzymes such as hyaluronidase and free radicals. Furthermore, HA is soluble in water at room temperature, which can also make it less suited to certain applications.

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Various attempts have therefore been made to prepare more stable forms of HA, in particular by cross-linking the HA molecules. Hydroxyl groups may be cross-linked via an ether linkage and carboxyl groups via an ester linkage. HA may be cross-linked at pH levels less than 9 at which ester bonds will form via carboxyl groups or at pH levels greater than 9 at which ether bonds will form via hydroxyl groups. Ether bonds, in contrast to ester bonds, are relatively resistant to physiological degradation.

30

US Patent No 4,582,865 (Biomatrix Inc) describes the preparation of cross-linked gels of hyaluronic acid which are formed by cross-linking HA either by itself or mixed with other hydrophilic polymers using divinyl sulfone as the cross-linking agent. It appears that in this case the cross-linking occurs via the hydroxyl groups of HA. There is no disclosure or teaching of drying hyaluronic acid gels after the addition of cross-linking agent and prior to washing out excess agent in this method.

US Patent No 5,827,937 (Agerup) describes polysaccharide (which may be inter alia hyaluronic acid) gel compositions which are prepared by forming an aqueous solution of the polysaccharide, initiating cross-linking in the presence of a polyfunctional cross-linking agent, sterically hindering the cross-linking reaction from being terminated before gelation occurs (eg by diluting the solution) and then reintroducing sterically unhindered conditions (eg by evaporating the solution) so as to continue the cross-linking to a viscoelastic gel. The cross-linking in this method may be performed under alkaline or acidic conditions. There is no disclosure or teaching of drying (removing the solvent) the cross-linked hyaluronic acid in this method.

WO 00/46253 (Fermentech Ltd) describes hyaluronic acid cross-linked with other polymers by two different types of cross-linking bonds, to effect a 'double cross-linking'. The formation of different types of bonds is achieved by effecting the cross-linking via different functional groups. Thus, for example, one type of bond may be formed by cross-linking via hydroxyl groups and a different functional bond formed by cross-linking via e g carboxyl groups. Cross-linking of HA with different functional bonds is the essence of this method.

WO 87/07898 there discloses a reaction of a polysaccharide with a polyfunctional epoxide, removal of excess of epoxide and finally the employment of a drying operation to cross-link said polysaccharide into a film, powdered material or similar dry product. However, there is no suggestion of maintaining alkaline conditions to induce the formation solely of ether bonds.

US Patent No 4,963,666 (Pharmacia) describes a process whereby polysaccharide is monosubstituted by a cross-linking agent at low concentration under alkaline conditions to form ether linkages. The mixture is washed to pH 5.5 inducing some ester linkages and then, in one example, concentrated by slow evaporation to complete cross-linking by forming ester linkages. In another example, the pH is increased following washing by the addition of ammonia. This is followed by slow evaporation to complete the cross-linking process to give mainly ether linkages with

some ester linkages. There is no disclosure or teaching of maintaining alkaline conditions throughout the entire process thereby avoiding the formation of ester linkages.

5 The present inventors have developed cross-linked polysaccharide compositions capable of forming a hydrogel having viscoelastic properties and tissue persistence.

Disclosure of Invention

10 It has unexpectedly been found that when cross-linked hyaluronic acid gel is dried before washing whilst retaining alkaline conditions the hyaluronic acid cross-linked in such a manner has greater resistance to degradation than hyaluronic acid cross-linked by other methods. Hyaluronic acid cross-linked in this way will contain ether bonds which, in contrast to ester bonds formed at pH levels less than 9, are more resistant to physiological degradation.

15 In a first aspect, the present invention provides a process for preparing a swellable biocompatible cross-linked polysaccharide composition, the process comprising:

- (a) forming an alkaline aqueous solution of a water soluble, cross-linkable polysaccharide;
 - (b) adding a poly-functional cross-linking agent to the aqueous solution to form a reaction mixture and incubating the reaction mixture under conditions to allow substantially ether cross-linking between the agent and the polysaccharide; and
 - (c) obtaining a swellable cross-linked polysaccharide composition without using acidification.
- 20

In a preferred form, the process further includes:

- (d) washing the cross-linked polysaccharide composition to remove any uncross-linked poly-functional cross-linking agent.
- 25

30 Preferably, the cross-linked polysaccharide composition according to the present invention is substantially resistant to enzymatic degradation under physiological conditions. More preferably, the cross-linked polysaccharide composition according to the present invention is substantially resistant to hyaluronidase degradation.

By the term 'swellable', it relates to the ability of the composition to absorb water within the matrix due to its poly-electrolyte properties thereby achieving much greater volumes than the dry material.

Preferably, the cross-linked polysaccharide composition when formulated in solution or gel form is relatively resistant to degradation following extrusion through a narrow gauge needle. Extrusion through a needle can break many gel types into smaller particles if they are not resistant to this type of shear stress. More preferably, the composition is relatively resistant to degradation following extrusion through a 30 gauge needle. The compositions when formulated in solution or gel form are particularly suitable for injection into tissue or skin without substantial loss of the structural integrity of the solution or gel.

Preferably, the polysaccharide is selected from naturally-occurring carboxylate-containing polysaccharides, such as hyaluronic acid, pectin, xanthan, alginic acid, or anionic derivatives of neutral polysaccharides such as carboxymethyl cellulose, carboxymethyl dextran or carboxymethyl starch. More preferably the polysaccharide is hyaluronic acid.

Preferably, the alkaline conditions have a pH within the range of about 9 to 12. More preferably, the pH is around 9. Preferably, sodium hydroxide is used to provide the alkaline conditions.

Preferably, the poly-functional cross-linking agent is selected from aldehydes, epoxides, polyaziridyl compounds, glycidyl ethers, divinyl sulphones or mixtures thereof. Suitable epoxy-type cross-linking agents include bi- or poly-functional epoxides, such as lower aliphatic epoxides or their corresponding epihalohydrins. Such agents that are well known to persons skilled in the art include 1,4-butanediol diglycidyl ether (BDDE), 1,2-ethanediol diglycidyl ether, epoxy-substituted pentaerythritol (e.g. SHELL 162) and epihalohydrins thereof. More preferably, the poly-functional cross-linking agent is 1,4-butanediol diglycidyl ether.

Preferably, the reaction is carried out with concentrations of the polysaccharide within the range of about 1 to 5% (w/v). More preferably, the polysaccharide is used at a concentration of about 4% (w/v).

The concentration of sodium hydroxide may be within the range of about 0.1 to 1% (w/v). Preferably, hydroxide is used at a concentration of about 1%.

Preferably, the cross-linking agent is be used within the range of about 0.05 to 0.5%. More, preferably, the cross-linking agent is used at a concentration of about 0.1%.

Preferably, the cross-linked polysaccharide is obtained by drying the reaction mixture. The reaction mixture may be dried by any suitable means.

In one preferred form, a biologically active substance is enclosed within the cross-linked polysaccharide composition during the process to form a biologically active cross-linked polysaccharide composition. The biologically active substance can be hormones, cytokines, vaccines, cells, tissue augmenting substances, or mixtures thereof. In this form, in use, the composition forms a sustained release composition or a depot.

Preferably, the tissue augmenting substance comprises a polymer selected from collagen, starch, dextranomer, polylactide, poly-beta-hydroxybutyrate, or copolymers thereof.

10 In a second aspect, the present invention provides a swellable biocompatible cross-linked polysaccharide composition obtained from the process according to the first aspect of the present invention.

Preferably, the swellable biocompatible cross-linked polysaccharide composition is gel 60-1 or gel 60-2 defined herein.

15 In a third aspect, the present invention provides a swellable biocompatible cross-linked polysaccharide composition which is obtainable by cross-linking of a cross-linkable polysaccharide with a poly-functional cross-linking agent without acidification, wherein the cross-linked polysaccharide is substantially resistant to enzymatic degradation under physiological conditions and is relatively resistant to degradation following extrusion through a narrow gauge needle.

20 Preferably, the swellable biocompatible cross-linked polysaccharide composition is gel 60-1 or gel 60-2 defined herein.

The swellable biocompatible cross-linked polysaccharide composition can also comprise a biologically active substance enclosed therein. In this form, the swellable biocompatible cross-linked polysaccharide is useful as a sustained release composition or a depot preparation.

25 The swellable biocompatible cross-linked polysaccharide compositions according to the present invention are particularly suitable for use in tissue augmentation, for use in treating arthritis, for use in treating tissue adhesions, and for use in coating mammalian cells to reduce their immunogenicity,

30 In a fourth aspect, the present invention provides a method of medical or prophylactic treatment of a mammal, the method comprising administering a swellable biocompatible cross-linked polysaccharide composition according to the second or third aspects of the present invention to an animal in need of such a treatment.

Preferably, the animal is a mammal, more preferably a human. The medical or prophylactic treatment includes, but is not limited to, cosmetic and corrective implants, hormone replacement therapy, hormone treatment, contraception, joint lubrication, and ocular surgery.

5 In a fifth aspect, the present invention provides use of a swellable biocompatible cross-linked polysaccharide composition according to the second or third aspects of the present invention in the manufacture of a medicament for medical, prophylactic treatment or therapy.

10 In a sixth aspect, the present invention provides use of a swellable biocompatible cross-linked polysaccharide composition according to the second or third aspects of the present invention in methods of medical or prophylactic treatment or therapy.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following drawings and examples.

25

Brief Description of the Drawings

Figure 1: Titration curve of hyaluronidase on hyaluronic acid substrate.

Figure 2: Comparison of uronic acid (UA) release between gels 60-1 and 60-2.

Figure 3: UV absorption of UA in gels after 1 day.

30 Figure 4: UV absorption of UA at 530 nm at one, two and twelve days.

Figure 5: UV absorption at 530 nm, after two days incubation.

Figure 6: Comparison between gel 60-1, Restylane™ and Perlane™.

Mode(s) for Carrying Out the Invention

EXPERIMENTAL

Work carried out has shown that viscoelastic gels with ether cross-links can be prepared under conditions that minimise degradation of the hyaluronic acid (HA) chains.

- 5 A refined method is described that is reproducible. Assessment of the gels for resistance to hyaluronidase was studied using literature procedures but as most assay methods reported were applied to highly cross-linked, firm gels some refinement of the procedures was required.

10 **Synthesis of gels 60-1 (HTL) and 60-2 (Fluka)**

General method

- Separate samples of HTL (fibrous) and Fluka (powder) hyaluronic acid (0.5 g) were each dissolved in 1% NaOH (12.5 ml) with vigorous stirring over a period of 1 h. BDDE (1,4-butanediol diglycidyl ether) (12.5 μ l) was added with vigorous stirring for 5
- 15 min and then the resulting solution was incubated without stirring in a water bath at 45°C for 2 h. At the end of the incubation period the mixture was removed from the bath, stirred vigorously for 1 min and then water was removed under high vacuum over 1.5 h at 35–40°C. The resulting transparent gums were treated with isopropyl alcohol and water (IPA/H₂O) (6:4, 25 ml) for 22 h, and then the IPA/H₂O was replaced two more
- 20 times every 22 h, i.e. the gums were allowed to stand under IPA/H₂O (6:4, 3 \times 25 ml) over 66 h. After this time, the IPA/H₂O was removed and 1.3% acetic acid in water (25 ml) was added with stirring. After 35 min both samples had produced fully swollen gels with the HTL gel being noticeably more viscous than the Fluka gel. The gels were washed with IPA/H₂O (50 ml), IPA/H₂O (6:4, 25 ml), IPA/H₂O (8:2, 100 ml), and then IPA
- 25 (50 ml). The resulting opaque rubbery materials were then freeze dried to give opaque hard sheets. The dried material was reconstituted in freshly prepared phosphate buffered saline (PBS) over 24 h at 20 mg/ml for use in the following experiments. Gel 60-1 was sieved through a 500 μ m mesh while gel 60-2 was sieved through a 300 μ m mesh. The gels were used over a 3 month period and not found to deteriorate during
- 30 storage.

Carbazole Assay

As described in the literature, the reaction of uronic acids with carbazole is the most satisfactory method to estimate the quantity of uronic acids in different compounds. We have followed the procedure described by Bitter and Muir [T. Bitter and H. M. Muir, 5 *Anal. Biochem.* 4, 330-334 (1962)] to establish a typical standard curve.

Experimental method

Reagents:

- A: 0.025 M sodium tetraborate 10 H₂O in sulfuric acid 98%
- 10 B: 0.125% carbazole in absolute ethanol (stable 12 weeks at 4°C in the dark)
- C: 11 glucuronolactone solutions of 0;1;5;10;15;20;25;30;40;50;75 and 100 µg/ml in deionized water saturated with benzoic acid (stable for 6 months at 4°C).

In a typical procedure, 5 ml of reagent A were placed in a tube and cooled to – 15 70°C. One ml of solution C was carefully added. The tube was sealed and allowed to warm to room temperature. The tube was then well shaken and heated for 10 minutes in a vigorously boiling water bath. It was then cooled to room temperature. Aliquots (0.2 ml) of the reagent B were then added. The tube was shaken and heated again for 15 minutes. After returning to room temperature, the UV absorption was measured at 20 530 nm. By plotting the UV absorption values as a function of the concentration of glucuronolactone a titration curve was obtained (FIGURE 1):

Resistance to hyaluronidase of gels 60-1 (HTL) and 60-2 (Fluka)

In order to determine the concentration of uronic acid (UA) released by 25 hyaluronidase from the different gels we have followed the procedure described by X.B. Zhao *et al* who synthesized, characterized and studied the biostability *in vitro* of different cross-linked HA materials [X.B. Zhao, J.E. Fraser, C. Alexander, C. Lockett, B.J. White, J. Mat. Science, Materials in Medicine 13, 11-16 (2002)]. After various tests and some modifications to this method, we were able to test gels 60-1 and 60-2 at 20 mg/ml and 30 15 mg/ml concentration by incubating them with hyaluronidase according to the procedure described below.

Experimental method

One ml of each gel (60-1 20 mg, 60-1 15 mg, 60-2 20 mg and 60-2 15 mg) was suspended in 6 ml of PBS (pH = 7.4) containing 1 mg of hyaluronidase (containing 1010 U) and incubated at 37°C. After 5 days, 0.5 ml of each tube was diluted in 2 ml of isopropanol. The remaining gel, which was not destroyed by the enzyme, was precipitated and removed by centrifugation over 30 minutes. The supernatant liquids containing the uronic acid were then heated in a vigorously boiling bath of water during 30 minutes to denature the enzyme, and centrifuged again for 30 minutes to eliminate the enzyme. The volume of each tube was adjusted to 3.5 ml. The concentration of UA released by hyaluronidase was determined from the titration curve by measuring the absorption at 530 nm.

The different UV values can be compared together as seen in Figure 2.

Lower concentrations of UA were observed in gels containing less biopolymer (ie. 60-1 20 mg compared to 60-1 15 mg). Also gel 60-1 was significantly less degraded than gel 60-2 both at 15 and 20 mg.

The concentration of UA (in µg/ml of gel solution) after 5 days incubation was determined from the titration curve (Figure.1). A dilution factor of 7 (i.e. 3.5/0.5) needs to be taken into account as the 0.5 ml sample is diluted to a volume of 3.5 ml for the analysis.

[UA]: concentration of UA in the gel supernatant

[UA_{dil}]: concentration of UA deduced from the titration curve:

$$y = 0.0172[UA_{dil}] + 0.0215$$

$$[UA_{dil}] = (y - 0.0215) / 0.0172 \text{ where } y = \text{maximum absorption value at 530 nm}$$

$$[UA] = [UA_{dil}] \times 7 = [(y - 0.0215) / 0.0172] \times 7$$

60-1 20 mg: $y=0.439$, $[UA]= 170 \mu\text{g/ml}$

60-1 15 mg: $y=0.3515$, $[UA]= 134 \mu\text{g/ml}$

60-2 20 mg: $y=0.559$, $[UA]= 219 \mu\text{g/ml}$

60-2 15 mg: $y=0.539$, $[UA]= 211 \mu\text{g/ml}$

Comparison of gel 60-1 with commercial gels Restylane™ and Perlane™

The comparison between gel 60-1 and commercial gels Restylane™ [Q-Med AB, Uppsala, Sweden] and Perlane™ [Q-Med AB, Uppsala, Sweden] was performed on a smaller scale and over a shorter period. This required adjustments to the previous procedure, as described below.

Experimental method

Sample (0.4 ml) of each gel was suspended in 3 ml of PBS (pH = 7.4) containing 0.5 mg of hyaluronidase (505 U) and incubated at 37°C. The tested gels were: Restylane™ at 20 and 15 mg/ml, Perlane™ at 20 and 15 mg/ml and Gel 60-1 at 20 and 15 mg/ml. After 1 day, 0.25 ml of each tube was diluted in 2 ml of isopropanol. The residual gel, which was not destroyed by the enzyme, was precipitated and removed by centrifugation over 30 minutes. Each tube was then heated in a vigorously boiling bath of water during 30 minutes to denature the enzyme, and centrifuged again for 30 minutes to eliminate the enzyme. The volume of each tube was adjusted to 2 ml. The concentration of UA released by hyaluronidase was determined from the titration curve by measuring the absorption at 530 nm. The UV absorbance curve at day 1 for each gel is represented in Figure 3.

For both the 15 mg/ml and 20 mg/ml series, gel 60-1 showed a much lower degradation (lower concentration of UA released), followed by Perlane™, and then Restylane™. It is important to note that even gel 60-1 at 20 mg/ml was less degraded than Perlane™ at 15 mg/ml.

Effect of needle size on gel deterioration

To determine the effect of the needle size on degradation of the gels, the procedure described above was repeated on Restylane™, Restylane™ extruded through a 32G needle, Perlane™, Perlane™ extruded through a 30G needle, gel 60-1 (500 µm), and gel 60-1 (500 µm) extruded through a 32G needle, and gel 60-1 (500 µm) extruded through a 30G needle. Gel concentration was fixed at 15 mg/ml.

A trial experiment was run in order to establish when the maximum level of degradation of the gels was obtained in the procedure conditions (0.15 g/l of hyaluronidase).

As the values obtained after two days were only slightly higher than those obtained after one day, a third set of measurements was taken after a much longer

period. Thus, after twelve days we noticed that the release of UA was very low compared to the first 48 hours, where the gels were mostly degraded (see Figure 4).

A two day incubation period was therefore sufficient to establish a comparison between the UA release (ie. degradation) of the different gels. In the Figure 5, the UV absorption at 530 nm, after two days, is represented for each experiment.

UV maxima and UA concentrations are listed in Table 1.

Table 1

	Maximum absorption (at 530 nm)	[UA] in $\mu\text{g/ml}^*$
Gel 60-1	1.111	511
Gel 60-1, needle 30G	1.193	549
Gel 60-1, needle 32G	1.24	571
Restylane	1.482	683
Restylane, needle 32G	1.617	746
Perlane	1.302	600
Perlane, needle 30G	1.466	676

* dilution factor is $2/0.25=8$

The first observation is that, the degradation level increased with the decrease of the needle size. As outlined in Figure 6, gel 60-1 seems to be less affected by an extrusion through a 30G needle than is Perlane™. Instead, the effect of extrusion through a 32G needle is similar for Restylane™ and gel 60-1.

It is important to note that even when gel 60-1 is extruded through a 32G needle, the UA concentration remained below the values observed for both Perlane™ and Restylane™ without extrusion. The much better resistance of gel 60-1 is again confirmed.

Evaluation of the degree of degradation of the gels

Calculation of the maximum UA release concentration

The UA extraction was performed by refluxing the gel solutions in the presence of hyaluronidase for 1 hour. As sulfuric acid is suspected to digest the gels and release UA as well (from preliminary work), the acidic treatment (see carbazole assay

procedure, below) was applied to the 0.25 ml sample without centrifugation. Before analysis, the solution volume was adjusted to 2 ml.

The UA concentrations obtained from the UV spectra and the titration curve are presented in Table 2.

5

Table 2

	Maximum absorption (at 530 nm)	[UA] _{max} in µg/ml*
Gel 60-1	1.6979	784
Restylane	1.6985	784
Perlane	1.6826	777

* dilution factor is 2/0.25=8

10 The similarity in the calculated concentrations tends to indicate that a maximum degradation level has been reached.

Percentage UA released

Results from Table 1 and Table 2 allowed us to calculate the percentage of UA released in the experimental conditions listed below, relative to the "maximum UA release" that can be expected to measure for these gels:

15

$$\%UA = [UA] / [UA]_{\max} \times 100$$

Gels: 0.4 ml at 15 mg/ml

Hyaluronidase: 0.5 mg

20

Solvent: PBS, 3 ml

Table 3

	[UA] in $\mu\text{g/ml}^*$	%UA
Gel 60-1	511	65
Gel 60-1, needle 30G	549	70
Gel 60-1, needle 32G	571	73
Restylane™	683	87
Restylane™, needle 32G	746	95
Perlane™	600	77
Perlane™, needle 30G	676	87

CONCLUSIONS

An optimum procedure for the preparation of stable cross-linked HA gels has been devised. By diluting the reconstituted gels with PBS the appropriate viscosity can be achieved.

Hyaluronidase resistance studies showed that the new gels were superior to commercially available gels. It should be noted that the assay method was based on those used to test very dense hard gels rather than soft flowing gels. This meant that very likely the concentration of enzyme used was relatively high. Consequently, all gels were almost completely degraded after 2 days. Nevertheless, the 2 day window allowed a good comparison of all gels to be made.

The compositions according to the present invention form a swellable hydrogel which most likely has intra- and extra molecular ether cross-links which favour prolonged tissue persistence. The compositions are different from the prior art in that they have dense cross-links but are also swellable, allowing them to be used as a gel which can be injected through a needle.

Although a densely cross-linked HA can be prepared in one step without drying by using a large amount of cross-linking agent, the result is an unswellable material. Therefore, such a material cannot be swollen and used for injection through a needle.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this 17th day of April 2003

Clearcoll Pty Ltd

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ALLENS ARTHUR ROBINSON

PATENT & TRADE MARKS ATTORNEYS

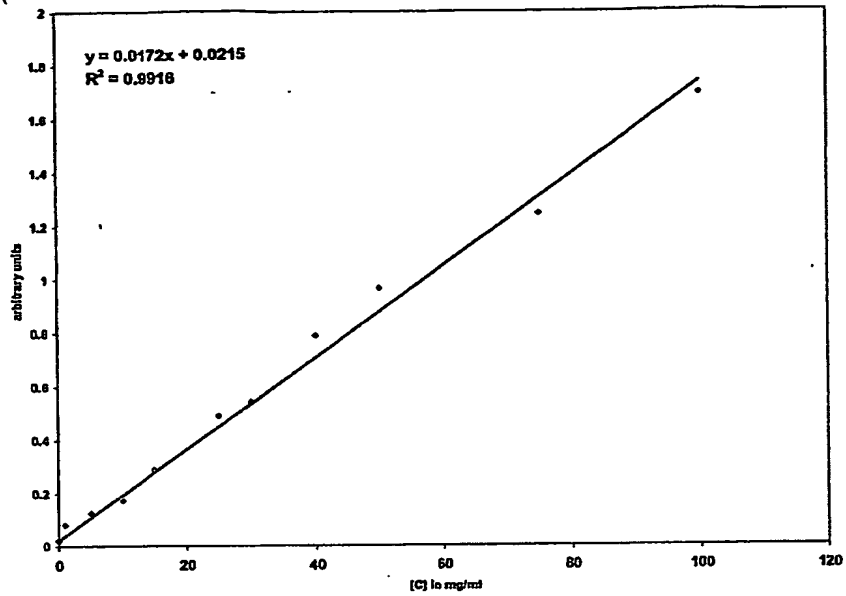


Figure 1: Titration curve

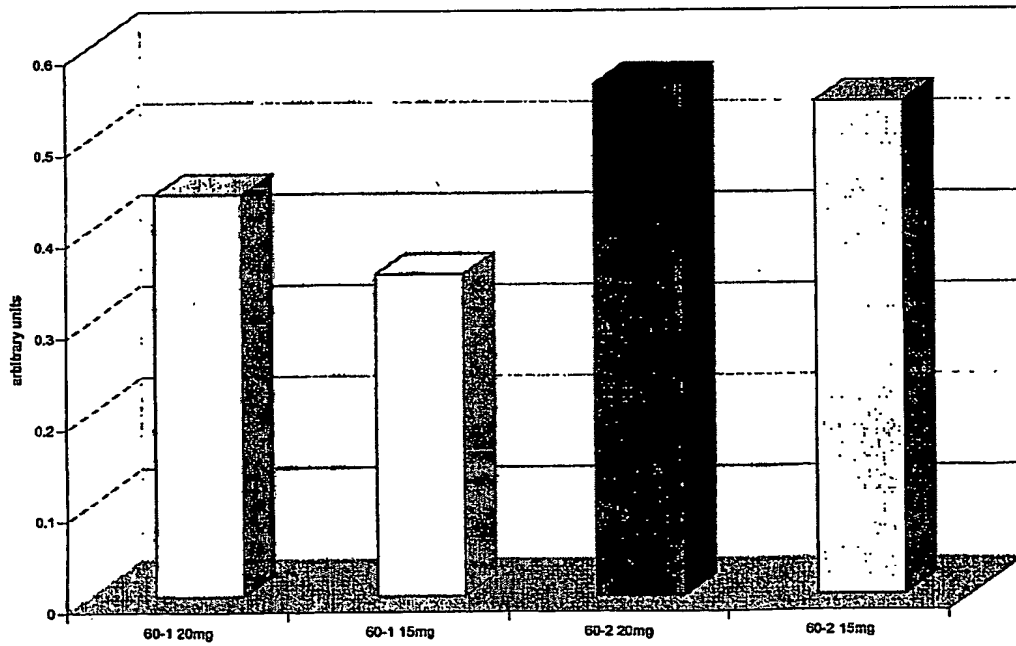


Figure 2: Comparison of UA release between gels 60-1 and 60-2

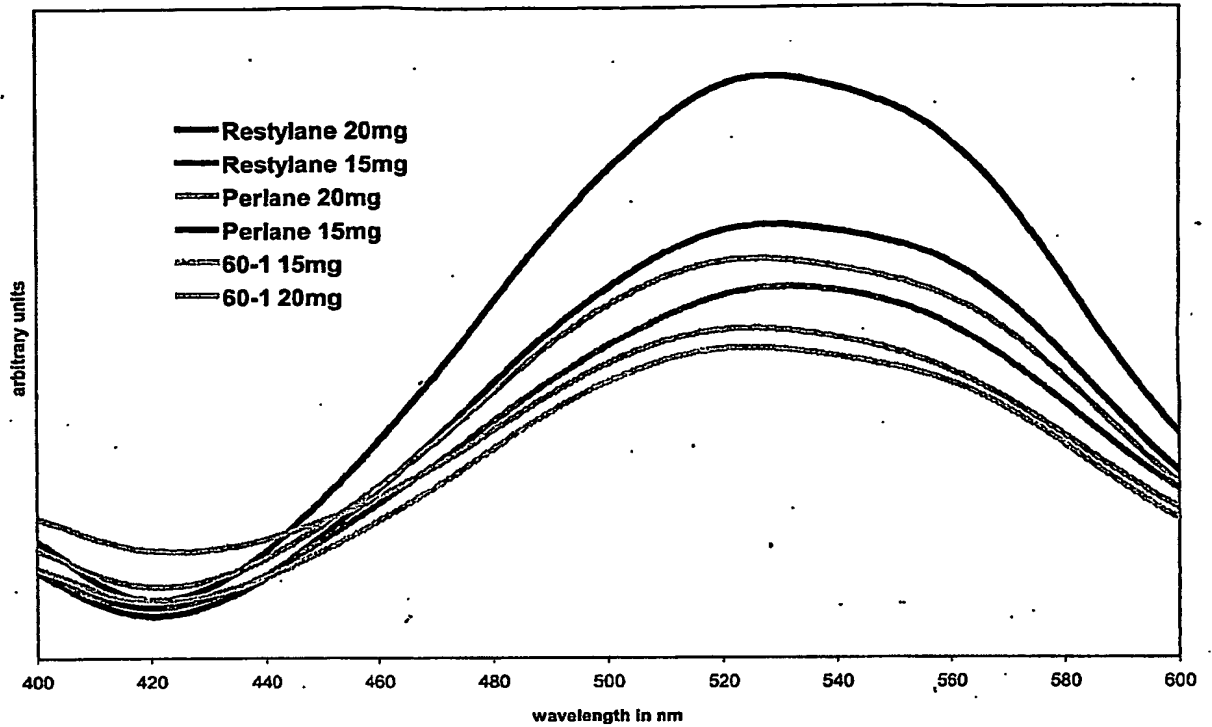


Figure 3: UV absorption of UA in gels after 1 day

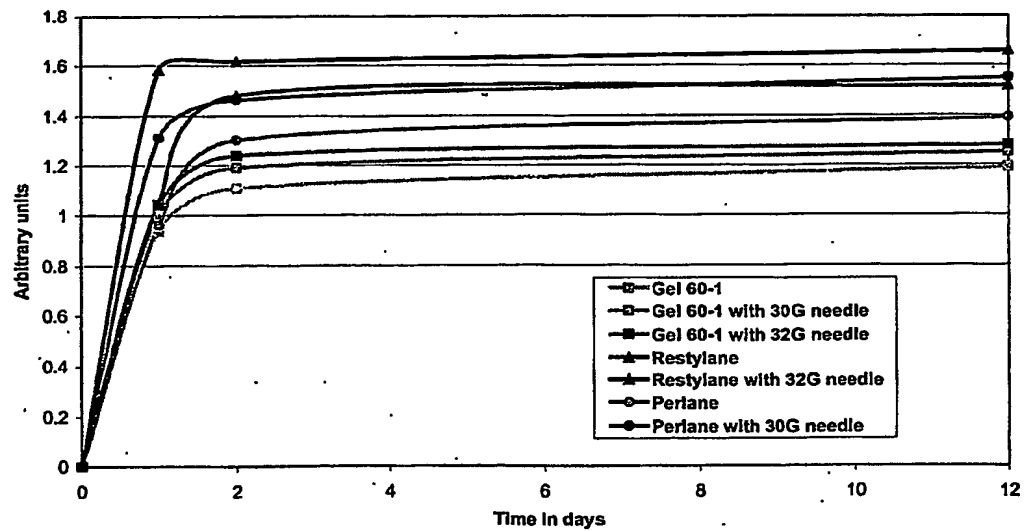


Figure 4: UV absorption of UA at 530 nm at one, two and twelve days

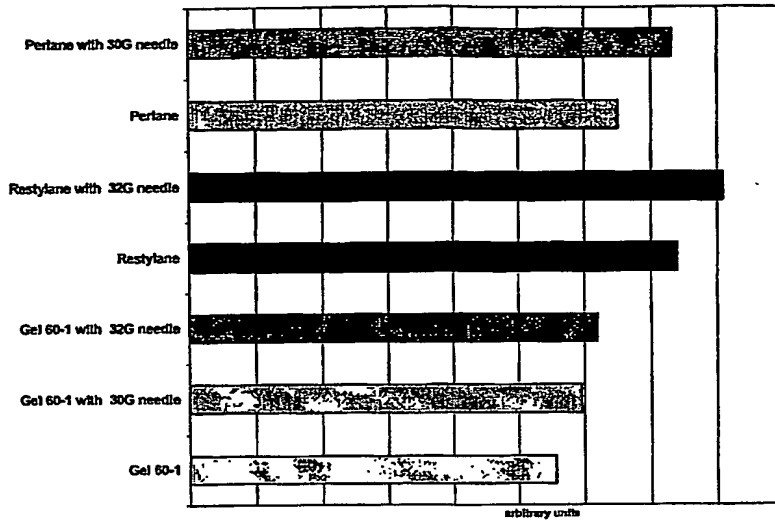


Figure 5: UV absorption at 530 nm, after two days incubation

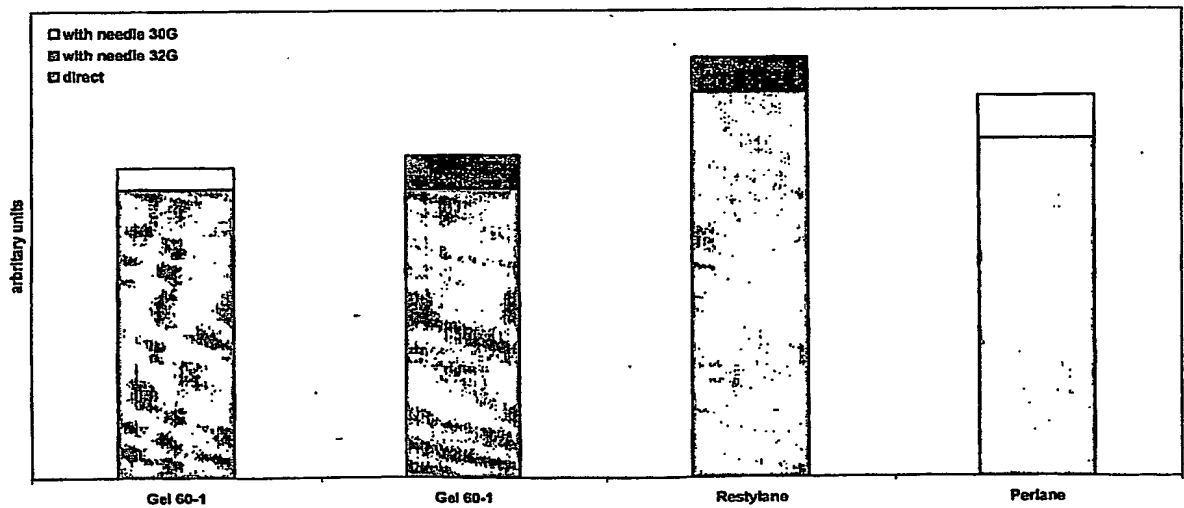


Figure 6: Comparison between gel 60-1, Restylane and Perlanc